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☐	L6	L5 and (rrna or r-rna or ribosomal$)	2
☐	L5	l4 or l3 or l2	60
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☐	L4	(RRN-O)!	12
☐	L3	(RRNO)!	48
☐	L2	(RRNO)!	48
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☐	L1	rrno near gene	0

END OF SEARCH HISTORY

Other Reference Publication (11):
Haima, Peter, et al., "Novel plasmid marker rescue transformation system for molecular cloning in Bacillus subtilis enableing direct selection of recombinants," Mol. Gen. Genet, vol. 223, pp. 185-181, 1990.

Other Reference Publication (23):
Perego, Marta, et al., "Sequence Analysis and Regulation of the hpr Locus, a Regulatory Gene for Protease Production and Sporulation in Bacillus subtilis," Journal of Bacteriology, vol. 170, No. 6, pp. 2560-2567, Jun., 1988.

Other Reference Publication (27):
Sonenshein, Abraham L., "Metabolic Regulation of Sporulation and Other Stationary-Phase Phenomena," Regulation of Procaryotic Development, Smith, I. et al, ed., American Society for Microbiology, Washington, D.C., pp. 109-130, 1989.

DOCUMENT-IDENTIFIER: US 6521440 B1
TITLE: Proteases from gram-positive organisms
Brief Summary Text (26):
In another aspect, the gram-positive host having one or more metallo-protease deletions or mutations is further genetically engineered to produce a desired protein. In one embodiment of the present invention, the desired protein is heterologous to the gram-positive host cell. In another embodiment, the desired protein is homologous to the host cell. The present invention encompasses a gram-positive host cell having a deletion, mutation or interruption of the nucleic acid encoding the naturally occurring homologous protein, such as a protease, and having nucleic acid encoding the homologous protein re-introduced in a recombinant form. In another embodiment, the host cell produces the homologous protein. Accordingly, the present invention also provides methods and expression systems for reducing degradation of heterologous proteins produced in gram-positive microorganisms. The gram-positive microorganism may be normally sporulating or non-sporulating. In a preferred embodiment, the gram positive host cell is a Bacillus. In another preferred embodiment, the Bacillus host cell is Bacillus. In another embodiment, the Bacillus is selected from the group consisting of B. subtilis, B. licheniformis, B. lentus, B. brevis, B. stearothermophilus, B. alkalophilus, B. amyloliquefaciens, B. coagulans, B. circulans, B. lautus and Bacillus thuringiensis.

Other Reference Publication (15):
Haima, Peter et al., "Novel plasmid marker rescue transformation system for molecular cloning in Bacillus subtilis enabling direct selection of recombinants," Mol. Gen. Genet. 223:185-191, 1990.

DOCUMENT-IDENTIFIER: US 6544792 B1
TITLE: Production of secreted polypeptides

Detailed Description Text (6):

Rudner, D. Z. et al., "The spo0K Locus of Bacillus subtilis Is Homologous to the Oligopeptide Permease Locus and Is Required for Sporulation and Competence" J. Bacteriol. vol. 173, No. 4, pp. 1388-1398.

Other Reference Publication (121):
Mathlopoulos et al., "A Bacillus subtilis dipeptide transport system expressed early during sporulation," Molecular Microbiology, 5(8): 1903-1913 (1991).

Other Reference Publication (126):
Mathiopoulos et al., "A Bacillus subtilis dipeptide transport system expressed early during sporulation," Mol. Microbio. (1991) 5(8), 1903-1913 (XP-000982568).

DOCUMENT-IDENTIFIER: US 6911322 B2
**** See image for Certificate of Correction ****
TITLE: Mutant aprE promoter
Brief Summary Text (5):
The aprE gene of B. subtilis codes for the extracellular protease subtilisn, a valuable enzyme manufactured by the biotechnology industry (Debadov V G (1982) The Industrial Use of Bacilli. In: Dubnau D A (ed) The Molecular Biology of the Bacilli. Academic Press: New York/London, vol 1, pp 331-370). The development of recombinant protein production systems using B. subtilis as a host organism, especially those driven by the subtilisin promoter, provides an important tool for research and commercial production in this area (Oyama et al. (1989) Secretion of Escherichia coli Aminopeptidase P in Bacillus subtilis using the Prepro-Structure Coding Region of Subtilisin Amylosacchariticus. J. Ferment. Bioeng. 68: 289-292). Although subtilisin synthesis is not required for sporulation (Stahl and Ferrari (1984) Replacement of the Bacillus subtilis Subtilisin Structural Gene With an In Vitro-Derived Deletion Mutation. J Bacteriol. 158: 411-418), its production is triggered by mechanisms common to those events responsible for the sporulation initiation, and hence, it has served as a model for developmentally-associated gene expression (Sonenshein A L (1989) Metabolic Regulation of Sporulation and Other Stationary-Phase Phenomenon. In: Smith I, Slepecky R A, Setlow P (eds) Regulation of Procaryotic Development. American Society for Microbiology, Washington, D.C. pp 109-130). The aprE gene is transcribed by sigma A (.sigma..sup.A) and its expression is highly controlled by several regulators, such as: DegU/DegS, AbrB, Hpr and SinR (Valle and Ferrari (1989) In: Smith I, Slepecky R A, Setlow P (eds) Regulation of Procaryotic Development. American Society for Microbiology. Washington, D.C. pp 131-146). A consensus sigma A promoter has been identified (Helman et al., 1995, Nucleic Acid Research, Vol. 23, pp. 2351-2360). In spite of advances in the understanding of production of proteins in host cells, there remains a need for methods for increasing expression of proteins in host cells, such as Bacillus host cells.

Detailed Description Text (21):
Plasmid pT7-aprE was constructed by the cloning of the 509 base pairs (bp) EcoRI-BamHI fragment derived from plasmid pSG35.1 (Ferrari E, Henner D J, Perego M, Hoch J A (1988) Transcription of Bacillus subtilis subtilisin and expression of subtilisin in sporulation mutants. J. Bacteriol. 170: 289-295), that contains the aprE promoter and the first eight codons of the structural gene, into the plasmid pT7 (Novagen). This new plasmid was used as a template for single or combinatorial oligonucleotide directed PCR mutagenesis according to the protocol described by Merino et al. (1992) A general PCR-based method for single or combinatorial oligonucleotide-directed mutagenesis on pUC/M13 vectors. Biotechniques 12: 509-510). PCRs were carried out with Taq DNA polymerase (Promega Co.) in a Perkin Elmer PCR System. The nucleotide substitutions introduced into the 5' aprE regulatory region were as follows: A-34.fwdarw.T, C-33.fwdarw.G, T-32.fwdarw.A, A-31.fwdarw.C, A-12.fwdarw.G, G+1.fwdarw.A. The final products of the PCR mutagenesis were verified by determining the nucleotide sequence using the dideoxy chain termination method described by Sanger et al. (1977). These DNAs were digested with EcoRI and BamHI, and cloned into the same restriction sites of the integrative plasmid pSG-PLK. Plasmid pSG-PLK is a pSG35.1 derivative in which the EcoRI-BamHI region has been replaced by the polylinker derived from pUC19, leaving a promoterless lacZ gene. This change in pSG-PLK provides an easier selection of transformants because colonies plated on X-Gal (5-bromo-4-chloro-3-indolyl-.beta.-D-galactopyranoside) agar are blue only if they carry the aprE promoter. Table 1 provides an index to the B. subtilis strains constructed.

Detailed Description Text (27):
It has been reported that the hpr2 mutation increases the expression levels of subtilisin. This mutation is a deletion of a DNA segment of 359 bp of the structural gene, that decreases 65% its activity (Perego and Hoch 1988 Sequence Analysis and Regulation of the hpr locus, a Regulatory Gene for Protease Production and Sporulation in Bacillus subtilis. J. Bacteriol. 170: 2560-2567). degU32 is a mutation that consists in the substitution A2006.fwdarw.T that changes an histidine residue to a leucine residue in the 12.sup.th amino acid of the protein (Henner et al. 1988 Localization of Bacillus subtilis sacU(Hy) mutations to two linked genes with similarities to the conserved procaryotic family of two-component signalling systems. J. Bacteriol. 170: 5102-9). This mutation increases the DegU-PO.sub.4 state, hence it carries out its activating effect for a longer time.

Detailed Description Text (35):
In order to have a direct estimation of the synthesis of the .beta.-galactosidase protein for establishing a direct relationship with the activity level found in our overproducer strains, we analyzed the total protein profile by SDS-PAGE of the strain JJ6 (BSR1 amyE::pTTGACA hpr2 degU32), the strain with the highest level of .beta.-galactosidase activity. Strain JJ6 was grown in Shaeffer medium and samples were taken at regular intervals. The cell-free extracts obtained by sonication were analyzed by SDS-PAGE and stained with Coomassie brilliant blue (FIG. 2). .beta.-galactosidase protein was observed as a band with a molecular mass of 116 kDa. The sporulation process started five hours

after the inoculation. At this time, the expression of the recombinant protein started and reached its maximum two hours later, corresponding roughly to 10% of the total intracellular protein.

Other Reference Publication (12):
*Haima, Peter, et al., "Novel plasmid marker rescue transformation system for molecular cloning in Bacillus subtilis enabling direct selection of recombinants," Mol. Gen. Genet, vol. 223, pp. 185-191, 1990.

Other Reference Publication (24):
*Perego, Marta, et al., "Sequence Analysis and Regulation of the hpr Locus, a Regulatory Gene for Protease Production and Sporulation in Bacillus subtilis," Journal of Bacteriology, vol. 170, No. 6, pp. 2560-2567, Jun., 1988.

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*Sonenshein, Abraham L., "Metabolic Regulation of Sporulation and Other Stationary-Phase Phenomena," Regulation of Procaryotic Development, Smith, I. et al, ed., American Society for Microbiology, Washington, D.C., pp. 109-130, 1989.